Abomasal Infusion of *Cis* or *Trans* Fatty Acid Isomers and Energy Metabolism of Lactating Dairy Cows[1,2,3]

G. A. ROMO,[4] D. P. CASPER,[5] R. A. ERDMAN,[4,6] and B. B. TETER[7]
University of Maryland, College Park 20742
Nutrient Conservation and Metabolism Laboratory, Livestock and Poultry Sciences Institute, Beltsville Agricultural Research Center-East, USDA-ARS, Beltsville, MD 20705

ABSTRACT

Diets for dairy cows that provide or induce formation of *trans* isomers of polyunsaturated fatty acids result in reduced percentages of milk fat. The effect of abomasal infusion of *trans*-$C_{18:1}$ fatty acid isomers on energy utilization by mature cows was determined. Six multiparous Holstein cows in midlactation had ad libitum access to a basal diet containing 50% forage and 50% concentrate. Treatments were 1) no infusion, 2) infusion of 630 g/d of a fat mixture high in *cis*-$C_{18:1}$ isomers (64% *cis*-$C_{18:1}$; 68% high oleic sunflower oil and 32% cocoa butter), and 3) infusion of 623 g/d of a fat mixture high in *trans*-$C_{18:1}$ isomers (42% *trans*-$C_{18:1}$; 90% partially hydrogenated soybean oil and 10% high linoleic safflower oil). The experiment was a replicated 3 × 3 Latin square design with 4-wk periods. Measurements of energy balance were made in open circuit respiration chambers during wk 4 of each period. Fat infusion increased milk production by 2.5 kg/d; apparent digestibility of DM, OM, energy, ADF, and ash by 1 to 4 percentage units; metabolizable energy by 11%; and NE_L of the diet by 15%. Milk fat percentage and yield were higher when cows were infused with *cis*-$C_{18:1}$ than when they were infused with *trans*-$C_{18:1}$ (4.12% and 1.41 kg/d vs. 3.15% and 1.06 kg/d, respectively). Infusion of fat increased milk production, but *trans*-$C_{18:1}$ reduced milk fat and energy output.

(**Key words**: *trans*-$C_{18:1}$ fatty acids, energy metabolism, milk fat depression)

Abbreviation key: **CIS** = fat mixture high in *cis*-$C_{18:1}$ FA isomers, **FA** = fatty acid, **ME** = metabolizable energy, **TRANS** = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

INTRODUCTION

Some of the changes in milk fat concentrations and yield have been related to the isomeric $C_{18:1}$ fatty acid (**FA**) composition of supplemental fats fed to dairy cows (16, 22, 29). Different fats, varying in source and FA composition, have been studied (10, 22, 23, 29). Milk fat synthesis was reduced when cows were fed rations that provided either the precursors that led to the formation of *trans*-$C_{18:1}$ FA in the rumen or were fed *trans*-$C_{18:1}$ FA directly (16, 25, 29). Regardless of the source of *trans*-$C_{18:1}$ FA, an inverse relationship exists between milk fat percentage and the concentration of *trans*-$C_{18:1}$ FA in milk fat (29).

Energy partitioning by different fat sources has been estimated in cows that were abomasally infused with fats (5) and measured directly in cows that were fed salts of long-chain FA (1). Fat supplements are calorie dense and increase the efficiency of utilization of metabolizable energy (**ME**) (1). Although *trans*-$C_{18:1}$ FA were not studied, rations causing milk fat depression decreased the efficiency of ME utilization for milk synthesis (27). The objectives of this study were to determine the effects of abomasal infusion of fat mixtures high in *cis* or *trans* isomers of $C_{18:1}$ FA on milk production and composition and to determine the fate of the energy that is spared by the reduction in milk fat percentages by infusion of *trans*-$C_{18:1}$ FA in lactating dairy cows.

MATERIALS AND METHODS

Experimental Design

All procedures for this study were carried out under Protocol R-92-17, approved by the University of Maryland Animal Care and Use Committee, and Pro-

Received June 19, 1995.
Accepted April 19, 1996.

[1]Scientific Article A6637, Contribution Number 8854 of Project Number C114 of the Maryland Agricultural Experiment Station. No endorsements are herein implied.

[2]This publication is a component of the NC-185 project, Metabolic Relationships in Supply of Nutrients for Lactating Cows.

[3]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

[4]Department of Animal Science, University of Maryland, College Park.

[5]Present address: Cargill Animal Nutrition Center, PO Box 301, Elk River, MN 55330-0301.

[6]Reprint requests.

[7]Department of Chemistry and Biochemistry, University of Maryland, College Park.

tocols R-92N-30 and 31, approved by the Beltsville Area Research Animal Committee. Six multiparous ruminally cannulated Holstein cows [mean age, 58 ± 3.5 mo and DIM, 115 ± 8 ($\bar{X}$ ± SEM)] were randomly assigned to treatments using a replicated 3 × 3 Latin square balanced for residual effects. Treatments were no infusion (control), abomasal infusion of a fat mixture that was high in *cis*-$C_{18:1}$ FA (**CIS**), and abomasal infusion of a fat mixture that was high in *trans*-$C_{18:1}$ FA (**TRANS**).

The CIS mixture consisted of high oleic sunflower oil and cocoa butter, and the TRANS mixture consisted of vegetable shortening and high linoleic safflower oil (Table 1). Mixtures were blended to have essentially the same FA profile, including the total $C_{18:1}$ FA, but differed in the proportion of $C_{18:1}$ FA isomers. Vegetable shortening and cocoa butter were heated until they became liquid (40 to 50°C) in a microwave oven before being mixed with the oils. Fat mixtures were prepared every 4 d, weighed in glass jars in 700-g aliquots, and stored at 4 to 10°C until infusion.

Cows were fed daily at 0800 and 2000 h for ad libitum consumption of a basal total mixed diet (50% forage and 50% concentrate, DM basis) throughout the 12-wk experimental period (Table 2). The total mixed diet was formulated to meet NRC (21) guidelines for milk production at 45 kg/d with 4.1% fat (Table 3). Silage DM content was determined daily, and diet adjustments were made weekly to maintain constant forage to concentrate ratios (DM basis).

Infusion of Fat Mixtures

A special type of infusion design was required to keep the fat mixtures at 45 to 50°C and to maintain liquidity. A water bath and a circulating water line (1.27-cm i.d., 1.59-cm o.d. Tygon® tubing; Baxter Scientific Prod., Columbia, MD) attached to a water pump (Little Giant Pump Co., Oklahoma City, OK) were used to circulate water continuously between a fat infusion peristaltic pump (Manostat, New York, NY) and the ruminal cannula in the cow. Within the water line, a fat infusion line (0.16-cm i.d., 0.32-cm o.d. Tygon® tubing for CIS, and 0.32-cm i.d., 0.64-cm o.d. Tygon® tubing for TRANS) was passed through the ruminal cannula, the rumen, omasum, and into the abomasum, where the line was maintained using a circular plastisol flange (8.5 cm diameter; Auburn Plastics, Chicago, IL).

Daily fat mixtures were infused for 16 to 18 h continuously (~0.65 g of fat/min) starting at 0700 h;

TABLE 1. Ingredient and long-chain fatty acid (FA) composition of fat infusion mixtures and the basal diet for cows receiving no infusion or abomasal infusions of fat mixtures containing *cis* or *trans* FA isomers.

Composition	Fat mixture[1]				Basal diet	
	CIS		TRANS			
	(%)					
Ingredient						
High oleic sunflower oil[2]	68					
Cocoa butter[3]	32					
Vegetable shortening[4]			90			
High linoleic safflower oil[5]			10			
	(g/100 g of total FA)					
FA	$\bar{X}$[6]	SEM	$\bar{X}$[6]	SEM	$\bar{X}$[7]	SEM
$C_{16:0}$	11.1	0.15	9.5	0.12	19.6	0.16
$C_{18:0}$	14.0	0.03	12.9	0.65	4.0	0.06
cis-$C_{18:1}$	63.5	0.09	26.4	0.20	24.0	0.27
trans-$C_{18:1}$			41.5	0.44	0.4	0.01
$C_{18:2}$	9.2	0.03	8.1	0.10	48.2	0.22
$C_{\geq 20:n}$[8]	1.6	0.04	1.3	0.05	1.9	0.06

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]Trisun 80 high oleic sunflower oil (SVO Enterprises, Eastlake, OH).

[3]Wilbur Chocolate Co. (Lititz, PA).

[4]Heavy duty vegetable frying shortening (Beoco, Kankakee, IL).

[5]Oilseeds International Ltd. (San Francisco, CA).

[6]n = 6.

[7]n = 9.

[8]Includes saturated and unsaturated FA.

infusion was interrupted for 2 h/d when cows exercised. The amount of infused fat was recorded, and the jar with the fat mixture was replaced at 0700 h daily. Patency and location of the infusion line inside the cow was confirmed every 3 d. Cows had infusion tubes in place but received no infusion during the control treatment.

Experimental Procedure

Each experimental period consisted of 4 wk, of which wk 1 was the preliminary period during which cows received the basal diet but no infusion. During wk 2 through 4, cows scheduled for CIS or TRANS received the infusion of fat mixtures. Weeks 2 and 3 were used for adjustment to FA infusions, and wk 4 was used for data collection.

Cows were housed in individual tie stalls equipped with rubber mats and bedded with sawdust in an isothermic environment (18°C). Cows were milked twice daily at 0630 and 1830 h. During wk 1 through 3 of each period, cows were turned out for 2 h of exercise at 1200 h. Body weights were recorded biweekly at 1100 h.

Measurements for energy balance were conducted during wk 4 of each period by indirect calorimetry in the open circuit respiration chambers at the USDA-ARS Beltsville Agricultural Research Center (15). Measurements included a 6-d total collection of feces, urine, orts, and milk; three measurements of CH_4 and CO_2 production and O_2 consumption were taken per day beginning on d 2 of excreta collection. Daily records included water intake, feed offered, fat infused, orts, milk production, body temperature, and heart rate. Heat production was calculated from respiratory exchange plus CH_4, and urine N output was calculated using the equation of Brouwer (4). Energy balance was calculated as described by Flatt and Tabler (14).

In each period, daily samples of ration, orts, feces, milk, and fat mixtures were refrigerated, and urine was frozen. Samples were composited at the end of the week to be analyzed for DM, N, and gross energy and then were frozen for later analysis.

Ruminal samples were obtained at 1030 and 1230 h on d 16 and 21 of infusion; each sample represented five 150- to 200-ml subsamples from different locations in the rumen. Half of each ruminal sample was frozen, and the other half was squeezed through four layers of cheesecloth so that ruminal fluid (50 ml) could be collected. The pH of the ruminal fluid was determined by glass electrode (Corning Science Products, Corning, NY). Then, fluid was acidified to pH <2 with 18 *M* H_2SO_4 and frozen in plastic tubes for VFA analysis.

TABLE 2. Ingredients of the basal diet for cows receiving no infusion or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid isomers.

Ingredient	(% of DM)
Corn silage	16.0
Alfalfa haylage	34.0
Ground corn	28.4
Soybean meal	16.6
Blood meal	1.5
Sodium bicarbonate	1.0
Dicalcium phosphate	0.8
Limestone	0.6
Selenized TM salt[1]	0.4
Dynamate®[2]	0.4
Magnesium oxide	0.25
Sodium selenite	0.08
Zinc oxide	0.004
Vitamin mix[3]	0.02

[1]Trace-mineralized salt contains >94% and <99% NaCl and ≥0.2% Mn, 0.1% Fe, 0.1% Mg, 0.05% Se, 0.025% Cu, 0.01% Co, 0.008% Zn, and 0.007% I.

[2]Pitman-Moore, Inc. (Chicago, IL). Guaranteed analysis: 22% S, 18% K, and 11% Mg.

[3]Contains 35% vitamin A (9,980,000 IU/kg), 20% vitamin D (14,970,000 IU/kg), and 45% vitamin E (44,000 IU/kg).

TABLE 3. Mean chemical composition of the basal diet for cows receiving no infusion or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid isomers.

Item	(% of DM)	
	$\bar{X}$[1]	SEM
CP	18.6	0.3
Ether extract	2.7	0.02
NDF	35.3	1.0
ADF	19.2	0.3
Lignin	3.8	0.1
Ca	0.74	0.02
P	0.47	0.004
Mg	0.31	0.004
K	1.50	0.02
Na	0.45	0.01
RUP[2]	6.7	
DM[3]	50.4	0.3

[1]n = 6.

[2]Calculated from NRC (21).

[3]Percentage of as fed.

Sample Analysis

Wet composite samples of ration, orts, fat mixtures, feces, milk, and urine were analyzed for gross energy by an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IL) and for N by the Kjeldahl procedure (2). Oven-dried composite samples of ra-

tion, orts, and feces were analyzed for ADF, NDF, and lignin by the method of Goering and Van Soest (17) and for ether extract (2), ash (2), and minerals (ration only) at the New York DHI Forage Testing Laboratory (Ithaca, NY).

Long-chain FA composition of freeze-dried samples of ration, fat mixtures, milk, and ruminal contents were determined by capillary column chromatography using an internal standard as described previously (16). Correction factors to quantify the overlapping area between *trans* and *cis* octadecenoic methyl esters were estimated by argentation TLC and GLC using a 100-m fused silica capillary column (0.25 mm i.d.) coated with SP-2560™ (Supelco Inc., Bellefonte, PA) (6).

Milk sample composites were analyzed for fat, protein, and SNF by infrared analysis and for SCC using a cell counter (Fossmatic 215; Foss Food Technology, Eden Prairie, MN) at a commercial laboratory (Environmental Systems Services, College Park, MD).

Ruminal VFA concentrations were determined in the ruminal fluid sample by GLC (Sigma 300; Sigma Chemical Co., St. Louis, MO) using ethylbutyrate as an internal standard in a 2-m glass column packed with GP 15% SP-1220™ and 1% H_3PO_4 on 100/120 Chromosorb® (Supelco Inc.) at 140°C.

Data Calculation and Statistical Analysis

The energy density of fat mixtures was calculated as the incremental addition of CIS or TRANS treatments compared with the control treatment for each cow. These calculations were made using intake energy, digestible energy, ME, and NE_L of the diets. The mean of the two collection times for pH of the ruminal fluid and mean ruminal VFA concentrations were used for analysis.

Mean daily responses during the week of data collection for each period were first analyzed as a crossover design to estimate residual effects of previous treatments within the Latin square. Residual effects were not significant ($P > 0.10$) and thus were dropped from the model. Effects of no infusion versus infusion of CIS or TRANS and effects of infusion of CIS versus TRANS were assessed using orthogonal contrasts. Analysis of variance was conducted using the PROC MIXED procedure of SAS (26); cow was the random variable. Data are presented as least squares means.

RESULTS AND DISCUSSION

Nutrient Intake

The DMI of the basal diet was lower ($P < 0.05$), and total DMI (basal diet and infusion) tended to be lower ($P < 0.10$), when cows received CIS or TRANS rather than the control treatment (Table 4). Reductions in DMI in cows infused with fat (5, 16) or in cows fed fats (1, 29) have been observed previously. In the present study, cows compensated for fat infusion by reducing DMI by an amount that was approxi-

TABLE 4. Characteristics of cows and intakes for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid (FA) isomers.

Item	Treatment[1]			SED[2]	
	Control	CIS	TRANS	Fat	Isomer
Characteristics of cows					
BW, kg	650	647	644	6.0	6.9
Body temperature, °C	38.6	38.7	38.8	0.07†	0.08
Heart rate, no. of beats/min	78.8	76.9	79.2	2.8	3.3
Intake					
Basal diet DM, kg/d	22.9	21.4	20.4	0.76*	0.88
Fat infusion, g/d	NI[3]	630	623		7.0
Total DM, kg/d	22.9	22.0	21.0	0.76†	0.88
Water, L/d	104.7	99.0	93.0	2.9**	3.3†

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

[3]NI = No infusion; cows had infusion tubing placed in the abomasum.

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

mately three times greater than the amount of fat infused (~2-kg reduction/~0.63 kg of infused fat), such that intake energy was similar among treatments. The amount of infused fat was not different between CIS and TRANS treatments. Water intake was lower ($P < 0.01$) during CIS and TRANS treatments than during the control treatment and tended to be lower ($P < 0.10$) for TRANS than for CIS. Lower water intakes were probably a reflection of lower ration intakes for cows infused with CIS or TRANS. No differences in BW, body temperature, or heart rate existed among treatments.

Abomasal Infusion

Abomasal infusion of FA allowed for the study of FA effects without the potential effects of ruminal fermentation. Successful delivery of fat mixtures to the abomasum was confirmed by patency of infusion lines and, indirectly, by the evaluation of ruminal major FA and pH (Table 5). Fat infusion did not affect either total or individual VFA in ruminal fluid or total or individual long-chain FA in ruminal contents, except for $C_{18:2}$ FA. Lower ruminal concentrations of $C_{18:2}$ FA and a numerical increase in $C_{18:0}$ for cows receiving CIS or TRANS ($P < 0.05$) probably reflected a more active biohydrogenation process because of the lower ration intake of cows receiving infused FA.

The advantages of our infusion design were that the infusion of fat sources that were solid at room temperature eliminated the need for a vehicle (e.g., meat solubles) (5), and our design allowed for continuous infusion. Continuous infusion more closely mimics the flow of ruminal digesta from the rumen to the abomasum than do pulse-dosed FA infusions. Pulse-dosed FA infusions alter fat digestion and absorption and have a greater influence on DMI (16).

Milk Production and Composition

Milk production was increased 2.5 kg/d ($P < 0.01$) by fat infusions (Table 6), but milk fat percentage was reduced ($P < 0.05$) by fat infusion. However, the reduction in milk fat concentration was because of TRANS infusion, which caused a reduction of 0.79 percentage unit ($P < 0.001$) compared with the milk fat reduction for cows infused with CIS. Absorption of

TABLE 5. Ruminal pH and VFA concentrations of fluid and long-chain fatty acid (FA) composition of ruminal contents for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* FA isomers.

	Treatment[1]			SED[2]	
Item	Control	CIS	TRANS	Fat	Isomer
pH	5.96[3]	5.98	6.01	0.05	0.06
Total VFA, m*M*	106	101	100	14	8
Acetate	63.2	59.7	60.1	7.8	4.5
Propionate	23.4	23.3	22.8	3.8	2.2
Isobutyrate	1.11	1.11	1.07	0.16	0.10
Butyrate	13.3	12.3	11.7	1.7	1.0
Isovalerate	2.19	1.97	2.18	0.27	0.15
Valerate	2.05	2.08	1.76	0.28	0.16*
Total FA, mg/g of contents	36.2	38.1	37.1	1.3	1.6
FA, g/100 g of total FA					
$C_{16:0}$	17.2	17.3	17.3	0.21	0.26
$C_{18:0}$	43.6	46.0	45.0	1.18	1.43
cis-$C_{18:1}$	8.75	8.16	8.11	0.31	0.38
trans-$C_{18:1}$	5.37	5.61	5.82	0.30	0.46
$C_{18:2}$	12.3	10.4	11.1	0.72†	0.87
$C_{\geq 20:n}$[4]	2.71	2.75	3.25	0.29	0.36
$C_{13:0} + C_{15:0} + C_{17:0} + C_{19:0}$	3.30	3.58	3.38	0.11	0.13

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

[3]n = 6; the mean of four samples (collected at 1030 and 1230 h on d 16 and 21) was used for each of the six cows.

[4]Includes saturated and unsaturated FA.

†$P \leq 0.10$.

*$P \leq 0.05$.

infused FA were confirmed through the increase in *trans*-$C_{18:1}$ FA in milk fat from 1.6% in the control and 1.7% in CIS to 14.0% in TRANS treatments. Because CIS and TRANS had opposite effects, the mean responses in milk fat and protein yields to fat infusion were not different; however, cows had lower fat and protein yields when infused with TRANS than when infused with CIS. Fat infusion did not affect 4 or 3.5% FCM, but both were lower ($P < 0.001$) during TRANS infusion than during CIS infusion. Milk protein and SNF percentages were lower ($P < 0.01$) when cows received CIS or TRANS treatments than when they were not infused. During TRANS treatment, SNF percentage was 0.13 percentage units lower ($P < 0.05$) than during CIS treatment.

Milk production responses to fat infusion that were similar to those observed in our study have been reported for cows supplemented with fat (1, 3, 8). Continuous infusion of TRANS resulted in lower milk fat percentage and yield, as has been reported in cows infused with pulse-doses of similar fat mixtures (16), cows fed fats containing *trans* FA (11), or cows fed supplements that provided precursors for the ruminal accumulation of *trans* FA (3, 29). The mechanism by which *trans* FA depressed milk fat is not known. From this experiment and that of Gaynor et al. (16), *trans* FA clearly were absorbed, and these FA exerted metabolic effects that were unrelated to changes in ruminal fermentation.

Total Tract Digestibilities of Nutrients

Apparent digestibilities of DM, OM, energy, ADF, and ash were increased by fat infusion (Table 7). Higher digestibilities of DM and energy would be expected because of the higher digestibility of FA than the remainder of the diet. Others have not found improvements in nutrient digestibilities, as measured by using markers, by fat supplementation (12, 29), or

TABLE 6. Milk production, composition of milk, and long-chain fatty acid (FA) composition of milk fat for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* FA isomers.

	Treatment[1]			SED[2]	
Item	Control	CIS	TRANS	Fat	Isomer
Milk, kg/d	31.7	34.5	33.9	0.6**	0.7
4% FCM, kg/d	31.1	34.9	29.4	0.9	1.0***
3.5% FCM, kg/d	33.4	37.5	31.6	1.0	1.1***
Fat, %	3.94	4.12	3.15	0.13*	0.15***
Protein, %	3.47	3.23	3.09	0.09**	0.10
Lactose, %	4.82	4.85	4.76	0.04	0.05†
SNF, %	9.01	8.79	8.57	0.08**	0.09*
Yield in milk, kg/d					
Fat	1.23	1.41	1.06	0.05	0.06***
Protein	1.08	1.11	1.04	0.02	0.02*
Lactose	1.53	1.67	1.61	0.04**	0.04
SNF	2.83	3.02	2.89	0.05*	0.06*
SCC, $\times 10^3$/ml	446	782	650	185	213
FA, g/100 g of total FA					
$C_{16:0}$	36.1	25.7	22.5	0.6***	0.7***
$C_{18:0}$	10.1	10.8	11.3	0.3	0.3**
cis-$C_{18:1}$	18.5	33.3	24.1	0.9***	1.1***
trans-$C_{18:1}$	1.56	1.73	14.02	0.46***	0.53***
$C_{18:2}$	2.49	4.81	5.11	0.17***	0.20***
$C_{\geq 20:n}$[3]	0.73	0.92	1.53	0.13	0.15***
$C_{13:0} + C_{15:0} + C_{17:0} + C_{19:0}$	4.92	3.31	4.39	0.12***	0.14**

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

[3]Includes saturated and unsaturated FA.

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.001$.

by infusion (16). In contrast, diets containing partially hydrogenated tallow have been shown to increase apparent digestibilities of CP and Ca but not apparent digestibilities of other nutrients (11). The use of a pulse-dose technique for fat infusion might have temporarily overwhelmed the absorptive capacity of the cow and thus failed to improve DM digestibility (16). In this experiment, apparent digestibilities of DM, OM, and ash were higher when cows received TRANS than when they received CIS. Fats have been reported (10, 18, 23) to have deleterious effects on microbial degradation of fiber. Therefore, the increase in apparent digestibilities of most dietary components with fat infusion and no infusion and with CIS probably were not related to fat infusion but to lower DMI.

N and Energy Utilization

Although cows had lower fecal ($P < 0.01$) and urinary N ($P < 0.001$) when receiving CIS or TRANS, N balance was not different for control cows because of similar milk N output among treatments and the lower ($P < 0.05$) N intake by cows that received fat infusion treatments (Table 8). Decreased N intake and unchanged milk N have been observed for cows fed diets supplemented with tallow and high oil corn (12). Fecal and milk N outputs were lower when cows were infused with TRANS than when cows were infused with CIS. Urine N was lower ($P < 0.05$), but milk N was higher ($P < 0.05$), as a proportion of intake N, when cows received CIS or TRANS infusions than when they received the control treatment. Therefore, although milk protein was lower during TRANS treatment, the efficiency of utilization of N for milk N deposition was improved by fat infusion, which was consistent with results of previous studies (1, 12, 28) in which fat was supplemented.

Intake energy was not different among treatments (Table 9). Both amount of energy and energy expressed as a proportion of total energy intake for feces, gas, and urine were lower when cows were infused with fat than when cows were not infused with fat. Milk energy tended to be higher ($P < 0.10$) for fat infusion treatments, but, given that TRANS reduced ($P < 0.001$) total milk energy, this difference was entirely due to higher ($P < 0.001$) milk energy during CIS treatment. Gaseous ($P < 0.05$), urinary ($P < 0.05$), and milk ($P < 0.001$) energies were lower, and fecal and heat ($P < 0.10$) energies tended to be lower, when cows were infused with TRANS than when they were infused with CIS. Lower estimated gaseous energy has been reported for cows fed diets supplemented with tallow and high oil corn (12) or

TABLE 7. Total tract apparent digestibilities (percentage) of dietary components for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid (FA) isomers.

	Treatment[1]			SED[2]	
Item	Control	CIS	TRANS	Fat	Isomer
DM	67.4	69.1	70.5	0.5***	0.6*
OM	68.5	70.1	71.4	0.5***	0.6*
N	68.4	68.6	70.8	1.3	1.5
Energy	67.1	70.1	70.8	0.6***	0.7
Neutral detergent solubles	79.7	80.0	80.4	0.8	0.9
NDF	50.3	51.6	54.3	1.5†	1.7
Hemicellulose	49.7	50.2	53.6	3.4	3.9
ADF	50.4	52.4	54.5	1.1*	1.3
Cellulose	56.3	58.3	60.6	2.5	2.9
Lignin	33.9	33.6	38.6	4.2	4.9
Ash	53.5	56.4	58.2	1.0**	1.1†
Total FA	75.9	81.3	77.9	1.6*	1.9†

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.001$.

TABLE 8. Partition of intake N for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid (FA) isomers.

	Treatment[1]			SED[2]	
N	Control	CIS	TRANS	Fat	Isomer
	(g/d)				
Intake	683	637	605	22*	25
Fecal	216	200	177	8**	9*
Urine	288	259	243	8***	9†
Milk	167	170	161	3	4*
Balance	10	6	23	13.1	15.1
	(% of intake)				
Fecal	31.6	31.4	29.2	1.27	1.47
Urine	42.2	40.8	40.1	0.78*	0.90
Milk	24.7	27.0	26.7	0.82*	0.95
Balance	1.3	0.6	3.8	2.08	2.40

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.001$.

TABLE 9. Partition of intake energy for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid (FA) isomers.

	Treatment[1]			SED[2]	
Energy	Control	CIS	TRANS	Fat	Isomer
	(Mcal/d)				
Intake	105.5	104.7	100.0	3.25	3.76
Fecal	34.7	31.5	29.2	0.96***	1.11†
Digestible	70.8	73.2	70.7	2.54	2.93
Gaseous	5.4	5.1	4.3	0.22**	0.25*
Urinary	4.1	3.8	3.6	0.09***	0.10*
Metabolizable	61.4	64.3	62.8	2.46	2.84
Heat	34.5	34.2	32.7	0.74	0.86†
Retained	26.9	30.1	30.0	2.55	2.94
Milk	22.5	25.4	22.0	0.64†	0.74***
Tissue	4.4	4.7	8.1	2.76	3.19
	(% of intake energy)				
Fecal	32.8	29.9	29.2	0.63***	0.73
Digestible	67.2	70.1	70.8	0.63***	0.73
Gaseous	5.1	4.8	4.3	0.26†	0.30
Urinary	3.9	3.6	3.6	0.08**	0.10
Metabolizable	58.1	61.6	62.8	0.86***	0.99
Heat	32.9	32.8	32.7	1.30	1.51
Retained	25.3	28.8	30.1	1.90*	2.19
Milk	21.5	24.4	22.1	1.03	1.19†
Tissue	3.7	4.3	8.0	2.78	3.21

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

†$P \leq 0.10$.

*$P \leq 0.05$.

**$P \leq 0.01$.

***$P \leq 0.001$.

partially hydrogenated tallow (11). When expressed as a percentage of energy intake, fecal, gaseous, urinary, and heat energy were not different. Digestible energy, ME, and retained energy were not different among treatments.

In general, partitioning of intake energy followed trends similar to those observed for cows fed supplemental Ca salts of long-chain FA (1). The reduction in fecal, gaseous, and urinary energies caused by fat infusion was not only because of lower DMI but also because of an increased utilization of gross energy for digestible energy (Table 9). Using the ME and NE_L contents of the control diet (Table 10), the gross efficiency of conversion of ME to NE_L was 60%. Using the calculated ME and NE_L averaged across fat treatments, the efficiency of conversion of ME to NE_L in the fat mixtures was 82%. This result agreed with the concept that the use of energy from fat for lactation is more efficient than the use of energy from other dietary components (1), as was shown in cows fed rations that were supplemented with tallow and high oil corn (12) and high grain rations (27). This theory might explain why TRANS produced milk fat depression but did not affect energetic efficiency. Differences in fecal, gaseous, urinary, heat, and milk energies between CIS and TRANS were attributable to lower intake energy because the partition of intake energy was similar whether cows received CIS or TRANS.

The fate of the energy that was not deposited in milk because of TRANS infusion could not be conclusively assessed from this experiment. The N balance and tissue energy balance were not affected by either fat infusion or the type of isomer, although both were numerically higher when cows received TRANS (Tables 8 and 9). The large variation associated with both numbers implied a wide range of responses in the cows. Variation in responses was probably random, but could have also been due to metabolic differences that were not accounted for in our calculations of heat production (19). Flatt et al. (13) have also noted that, for high producing cows fed different diets, varying the forage to concentrate proportions from 60:40 to 20:80 decreased milk energy as the proportion of forage in diet decreased, but body tissue energy remained unchanged. Milk energy decreased because, as the proportion of forage in diet decreased, milk fat yield and percentage decreased (13).

Influences of *cis* versus *trans* configuration of FA on energy utilization and fat metabolism have been found for nonruminants. Rats fed *trans,trans*-$C_{18:2}$ FA (10% of diet) had higher heat loss with similar heat expenditure and had lower ATP synthesis rates in liver mitochondria than did groups fed *cis* or *cis,trans* isomers (9); no differences were found in these parameters among rats fed *cis* and *trans* isomers of $C_{18:1}$. Our fat mixtures did not contain measurable amounts of *trans* isomers of $C_{18:2}$. In vitro cell culture

TABLE 10. Energy values of the diet and fat mixtures for cows receiving no infusion (control) or abomasal infusions of fat mixtures containing *cis* or *trans* fatty acid (FA) isomers.

Item	Treatment[1]			SED[2]	
	Control	CIS	TRANS	Fat	Isomer
	(Mcal/kg of DM)				
Diet[3]					
Intake energy	4.62	4.76	4.77	0.03***	0.03
Digestible energy	3.10	3.34	3.38	0.04***	0.05
Metabolizable energy	2.68	2.93	3.00	0.05***	0.06
NE_L	1.60	1.81	1.88	0.07**	0.08
Fat mixtures[4]					
Intake energy		9.61	9.67		0.08
Digestible energy		10.97	11.36		3.24
Metabolizable energy		11.01	12.34		3.01
NE_L		8.56	10.71		2.52

[1]CIS = Fat mixture high in *cis*-$C_{18:1}$ FA isomers; TRANS = fat mixture high in *trans*-$C_{18:1}$ FA isomers.

[2]SED = Standard error of the difference. Orthogonal contrasts: fat = no infusion versus infusion of CIS and TRANS, and isomer = infusion of CIS versus TRANS.

[3]Includes the basal diet and infused fat mixtures.

[4]Calculated by difference between the energy intake during control treatment and fat infusion treatments.

**$P \leq 0.01$.

***$P \leq 0.001$.

of adipocytes has shown a decreased accumulation of lipid in cells exposed to *trans* FA, probably because of induction of peroxisomal β-oxidation, which does not generate ATP (24). In addition, indirect measurements of storage and mobilization of lipids in adipose tissue also failed to detect differences in tissue deposition between cows infused with *cis* and *trans* isomers (16).

Results from this study show that the effects of *trans*-$C_{18:1}$ on lipid metabolism are not explained by consistent quantitative shifts in energy deposition among secretions and tissues. The use of labeled FA may be a more sensitive tool to trace the fate of *trans*-$C_{18:1}$. It is also possible that ruminants also experience the qualitative changes in energy metabolism that have been described for nonruminants.

Energy Values

The fat mixtures represented incremental additions and not substitutions to the control diet. Therefore, NE_L values for the fat mixtures were calculated by subtracting the energy value of the control diet from the CIS or TRANS diets for each cow. The NE_L content of the diet was increased ($P < 0.001$) by 0.25 Mcal/kg by fat infusion, and there was no isomer effect (Table 10). The digestible energy, ME, and NE_L were higher for cows receiving fat infusion treatments. However, the relative contribution of infused fat mixtures to the energy content of the diet in CIS and TRANS increased from 3.2 ± 0.6% in the intake energy value to a contribution of 15.8 ± 4% in the NE_L value of the total diet. The NE_L values of the fat infusion mixes were 8.56 Mcal/kg for CIS and 10.71 Mcal/kg for TRANS; these values were close to or exceeded the values for gross energy of the fats.

Utilization of ME might be different at different amounts of feed intake or physiological states (1). Thus, use of the energy values for the control diet to calculate energy might have ignored the possible interactions of period and treatment. Additive errors involved in calculations would explain values that were above the gross energy values, which have also been reported previously (1). Large errors arose because the infused fat was a small proportion (~3%) of the total DMI and because energy values for fat were calculated by difference (1, 18). Improper mixing and inherent errors in analysis of the diets and orts have been described as possible contributors to error (1). However, in this experiment, our infusion design allowed us to quantify precisely the amounts of fat infused (±20 g).

The ME and digestible energy values for the fat mixtures that exceeded the gross energy values indicated that there was an associative effect between fats and other dietary components such that separation of effects by difference overestimated the fat contribution (7). Most associative effects have been observed in digestible energy values (20). Our results suggested that associative effects existed in both digestible energy and in ME.

CONCLUSIONS

Continuous abomasal fat infusion increased the metabolizability of the diet, regardless of the isomeric differences. *Trans* FA differed from *cis* isomers in the way in which they affected energy deposition in milk. Neither the mechanism by which *trans* FA reduced milk fat nor the fate of the energy spared from milk fat was apparent from this experiment.

The similar characteristics of ruminal fermentation during fat infusion would imply a similar availability of VFA precursors for fat synthesis. Similar nutrient digestibilities between CIS and TRANS infusion would imply similar availability of absorbed nutrients for fat synthesis. Thus, TRANS might induce an isomer-specific uptake or metabolism of nutrients in certain tissues, specifically in the mammary gland and possibly in the adipose tissue.

REFERENCES

1 Andrew, S. M., H. F. Tyrrell, C. K. Reynolds, and R. A. Erdman. 1991. Net energy for lactation of calcium salts of long-chain fatty acids for cows fed silage-based diets. J. Dairy Sci. 74:2588.
2 Association of Official Analytical Chemists International. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.
3 Banks, W., J. L. Clapperton, A. K. Girdler, and W. Steele. 1984. Effect of inclusion of different forms of dietary fatty acid on the yield and composition of cow's milk. J. Dairy Res. 51:387.
4 Brouwer, E. 1965. Report of sub-committee on constants and factors. Page 441 *in* Proc. 3rd Symp. Energy Metab. K. L. Blaxter, ed. Publ. No. 11. Eur. Assoc. Anim. Prod., Troon, Scotland.
5 Christensen, R. A., J. K. Drackley, D. W. LaCount, and J. H. Clark. 1994. Infusion of four long-chain fatty acid mixtures into the abomasum of lactating dairy cows. J. Dairy Sci. 77:1052.
6 Christie, W. W. 1982. Lipid Analysis. 2nd ed. Pergamon Press, New York, NY.
7 Coppock, C. E., W. P. Flatt, and L. A. Moore. 1964. Effect of hay to grain ratio on utilization of metabolizable energy for milk production by dairy cows. J. Dairy Sci. 47:1330.
8 DePeters, E. J., S. J. Taylor, and R. L. Baldwin. 1989. Effect of dietary fat in isocaloric rations on the nitrogen content of milk from Holstein cows. J. Dairy Sci. 72:2949.
9 de Schrijver, R., and O. S. Privett. 1984. Energetic efficiency and mitochondrial function in rats fed *trans* fatty acids. J. Nutr. 114:1183.
10 Doreau, M., and A. Ferlay. 1994. Digestion and utilization of fatty acids by ruminants. Anim. Feed Sci. Technol. 45:379.
11 Drackley, J. K., and J. P. Elliott. 1993. Milk composition, ruminal characteristics, and nutrient utilization in dairy cows fed partially hydrogenated tallow. J. Dairy Sci. 76:183.

12 Elliott, J. P., J. K. Drackley, D. J. Schauff, and E. H. Jaster. 1993. Diets containing high oil corn and tallow for dairy cows during early lactation. J. Dairy Sci. 76:775.
13 Flatt, W. P., P. W. Moe, A. W. Munson, and T. Cooper. 1969. Energy utilization by high producing dairy cows. Page 221 *in* Energy Metabolism of Farm Animals. K. Blaxter, J. Kielanowski, and G. Thorbek, ed. Oriel Press, Newcastle Upon Tyne, United Kingdom.
14 Flatt, W. P., and K. A. Tabler. 1961. Formulae for computation of open circuit indirect calorimeter data with electronic data processing equipment. Page 39 *in* Proc. 2nd Symp. Energy Metab. Publ. No. 10. Eur. Assoc. Anim. Prod., Wageningen, The Netherlands.
15 Flatt, W. P., P. J. Van Soest, J. F. Sykes, and L. A. Moore. 1958. A description of the energy metabolism laboratory at the US Department of Agriculture, Agricultural Research Center, Beltsville, MD. Page 53 *in* Proc. 1st Symp. Energy Metab. Publ. No. 8. Eur. Assoc. Anim. Prod., Copenhagen, Denmark.
16 Gaynor, P. J., R. A. Erdman, B. B. Teter, J. Sampugna, A. V. Capuco, D. R. Waldo, and M. Hamosh. 1994. Milk fat yield and composition during abomasal infusion of *cis* or *trans* octanodecenoates in Holstein cows. J. Dairy Sci. 77:157.
17 Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.
18 Holter, J. B., H. H. Hayes, W. E. Urban, and A. H. Dutie. 1992. Energy balance and lactation response in Holstein cows supplemented with cottonseed with or without calcium soap. J. Dairy Sci. 75:1480.
19 Livesey, G., and M. Elia. 1988. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. Am. J. Clin. Nutr. 47:608.
20 Moe, P. W., W. P. Flatt, and H. F. Tyrrell. 1972. Net energy value of feeds for lactation. J. Dairy Sci. 55:945.
21 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.
22 Palmquist, D. L., A. Denise Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753.
23 Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations: review. J. Dairy Sci. 63:1.
24 Panigrahi, K., and J. Sampugna. 1993. Effects of *trans* fatty acids on lipid accumulation in 3T3-L1 cells. Lipids 28:1069.
25 Romo, G., R. Erdman, B. Teter, and D. P. Casper. 1994. Potential role of *trans* fatty acids in diet induced milk fat depression in dairy cows. Page 64 *in* Proc. Maryland Nutr. Conf. Feed Manuf., Baltimore. Univ. Maryland, College Park.
26 SAS® Technical Report P-229, SAS/STAT® Software: Changes and Enhancements, Release 6.07. 1992. SAS Inst., Inc., Cary, NC.
27 Tyrrell, H. F., and P. W. Moe. 1972. Net energy value for lactation of a high and low concentrate ration containing corn silage. J. Dairy Sci. 55:1106.
28 van der Honing, Y. 1979. The utilization by high-yielding cows of energy from animal tallow or soya bean oil added to a diet rich in concentrates. Page 315 *in* Proc. 8th Symp. Energy. Metab. L. E. Mount, ed. Publ. No. 26. Eur. Assoc. Anim. Prod. Butterworths, London, England.
29 Wonsil, B. J., J. H. Herbein, and B. A. Watkins. 1994. Dietary and ruminally derived *trans*-18:1 fatty acids alter bovine milk lipids. J. Nutr. 124:556.